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Apoptin-associating protein

Abstract

The invention relates to the field of apoptosis. The invention provides novel therapeutic possibilities, for example, novel combinatorial therapies or novel therapeutic compounds that can work alone, sequentially to, or jointly with Apoptin, especially in those cases wherein p53 is (partly) nonfunctional.

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Jan 17, 2000	EP	00200169.1
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Claims

1. An isolated or recombinant nucleic acid or functional equivalent or functional fragment thereof encoding an Apoptin-associating proteinaceous substance capable of providing apoptosis.

2. The isolated or recombinant nucleic acid of claim 1 derived from a cDNA library.

3. (Twice Amended) The isolated or recombinant nucleic acid of claim 2 wherein said cDNA library comprises human cDNA.

4. (Twice Amended) The isolated or recombinant nucleic acid of claim 1 capable of hybridizing to a nucleic acid molecule encoding the Apoptin-associating proteinaceous substance as shown in FIG. 1, 2, 5 or 7.

5. (Twice Amended) The isolated or recombinant nucleic acid of claim 1 being at least 60% homologous to a nucleic acid molecule, or to a functional equivalent or functional fragment thereof, encoding the Apoptin-associating proteinaceous substance as shown in FIG. 1, 2, 5 or 7.

6. (Previously Amended) A vector comprising the nucleic acid of claim 5.

7. The vector of claim 6 further comprising a gene-delivery vehicle.

8. (Previously Amended) A host cell comprising the nucleic acid of claim 1.

9. The host cell of claim 8 wherein said cell is a eukaryotic cell.

10. (Previously Amended) An isolated or recombinant Apoptin-associating proteinaceous substance capable of providing apoptosis encoded by the nucleic acid of claim 1.

12. (Previously Amended) The proteinaceous substance of claim 10 comprising at least a part of an amino acid sequence as shown in FIG. 3, 6 or 8 or a functional equivalent or functional fragment thereof.

13. (Twice Amended) An isolated or synthetic antibody specifically recognizing the proteinaceous substance or the functional equivalent or the functional fragment thereof according to claim 12.

15. (Twice Amended) A method of inducing apoptosis in a cell, said method comprising: introducing the proteinaceous substance of claim 12 to the cell in a manner to induce said apoptosis.

16. The method according to claim 15 wherein said apoptosis is p53-independent apoptosis.

17. (Previously Amended) The method according to claim 16 further comprising: administering or introducing a nucleic acid encoding Apoptin or a functional equivalent or fragment thereof, or Apoptin

or a functional equivalent or fragment thereof.

18. (Previously Amended) A pharmaceutical composition comprising a substance associated with Apoptin-associating proteinaceous substance, said substance comprising the nucleic acid of claim 1.

19. (Previously Amended) The pharmaceutical composition of claim 18 further comprising: a second nucleic acid encoding Apoptin or a functional equivalent or fragment thereof or Apoptin or a functional equivalent or fragment thereof.

20. (Previously Amended) The pharmaceutical composition of claim 18 wherein said substance is present in an amount sufficient to induce apoptosis.

21. (Previously Amended) The pharmaceutical composition of claim 20 wherein said apoptosis is p53-independent.

22. (Previously Amended) The pharmaceutical composition of claim 21, wherein the substance is present in an amount and manner for the treatment of a disease where enhanced cell proliferation or decreased cell death is observed.

23. (Amended) The pharmaceutical composition of claim 22 wherein said disease comprises cancer or an autoimmune disease.

24. (Twice Amended) A method for treating an individual carrying a disease where enhanced cell proliferation or decreased cell death is observed comprising treating said individual with the pharmaceutical composition of claim 18.

25. (Twice Amended) A method for detecting a presence of cancer cells or cells that are cancer prone in a sample of cells comprising transfecting cells in said sample with the nucleic acid of claim 1 and determining a percentage of apoptosis of cells in said sample.

26. (Twice Amended) A method for detecting a presence of cancer cells or cells that are cancer prone in a sample of cells comprising transfecting cells in said sample with the nucleic acid according to claim 1 and determining a intracellular localization of a protemaceous substance derived from said nucleic acid or a vector in said sample of said cells.

27. (Amended) The method according to claim 26 wherein a presence of said proteinaceous substance in said cells is detected by immunostaining said cells with an antibody.

29. (Twice Amended) A method for identifying a putative cancer-inducing agent, said method comprising: submitting a sample of cells to said agent, and detecting a presence of cancer cells or cells that are cancer prone in the sample of cells with the method according to claim 25.

30. The method according to claim 29 wherein said putative cancer-inducing agent comprises a genome or functional fragment thereof.

Description

[0001] Priority Claim: This application claims priority under 35 U.S.C. .sctn.119 of European Patent

applications EP 00250118.7, filed on Apr. 7, 2000 and EP 00200169.1, filed on Jan. 17, 2000, the contents of both of which are incorporated by this reference including all figures, claims and sequences identified therein.

BACKGROUND OF THE INVENTION

[0002] The invention relates to the field of apoptosis. Apoptosis is an active and programmed physiological process for eliminating superfluous, altered or malignant cells (Earnshaw, 1995; Duke et al., 1996). Apoptosis is characterized by shrinkage of cells, segmentation of the nucleus, condensation and cleavage of DNA into domain-sized fragments in most cells followed by internucleosomal degradation. The apoptotic cells fragment into membrane-enclosed apoptotic bodies. Finally, neighboring cells and/or macrophages will rapidly phagocytose these dying cells (Wylie et al., 1980; White, 1996). Cells grown under tissue-culture conditions and cells from tissue material can be analyzed for being apoptotic with agents staining DNA, for example, DAPI, which stains normal DNA strongly and regularly, whereas apoptotic DNA is stained weakly and/or irregularly (Noteborn et al., 1994; Telford et al., 1992).

[0003] The apoptotic process can be initiated by a variety of regulatory stimuli (Wyllie, 1995; White, 1996; Levine, 1997). Changes in the cell survival rate play an important role in human pathogenesis of diseases, for example, in cancer development and autoimmune diseases, where enhanced proliferation or decreased cell death (Kerr et al., 1994; Paulovich, 1997) is observed. A variety of chemotherapeutic compounds and radiation have been demonstrated to induce apoptosis in tumor cells, in many instances via wild-type p53 protein (Thompson, 1995; Bellamy et al., 1995; Steller, 1995; McDonell et al., 1995).

[0004] Many tumors, however, acquire a mutation in p53 during their development, often correlating with poor response to cancer therapy. Certain transforming genes of tumorigenic DNA viruses can inactivate p53 by directly binding to it (Teodoro, 1997). An example of such an agent is the large T antigen of the tumor DNA virus SV40. For several (leukemic) tumors, a high expression level of the proto-oncogene Bcl-2 or Bcr-abl is associated with a strong resistance to various apoptosis-inducing chemotherapeutic agents (Hockenberry 1994; Sachs and Lotem, 1997).

[0005] For such tumors lacking functional p53 (representing more than half of the tumors), alternative antitumor therapies are under development based on induction of apoptosis independent of p53 (Thompson 1995; Paulovich et al., 1997). One has to search for the factors involved in induction of apoptosis, which do not need p53 and/or can not be blocked by antiapoptotic activities, such as Bcl-2 or Bcr-abl-like ones. These factors might be part of a distinct apoptosis pathway or might be (far) downstream of the apoptosis inhibiting compounds.

[0006] Apoptin is a small protein derived from chicken anemia virus (CAV; Noteborn and De Boer, 1995; Noteborn et al., 1991; Noteborn et al., 1994, 1998a) which can induce apoptosis in human malignant and transformed cell lines, but not in untransformed human cell cultures. In vitro, Apoptin fails to induce programmed cell death in normal lymphoid, dermal, epidermal, endothelial and smooth-muscle cells. However, when normal cells are transformed they become susceptible to apoptosis by Apoptin. Long-term expression of Apoptin in normal human fibroblasts revealed that Apoptin has no toxic or transforming activity in these cells (Danen-van Oorschot, 1997 and Noteborn, 1996).

[0007] In normal cells, Apoptin was found predominantly in the cytoplasm, whereas in transformed or malignant cells, i.e., characterized by hyperplasia, metaplasia or dysplasia, it was located in the nucleus, suggesting that the localization of Apoptin is related to its activity (Danen-van Oorschot et al. 1997).